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**Supplementary Information**

# Sequence-independent, site-specific incorporation of chemical modifications to generate light-activated plasmids

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**DNA Sequences**

**Supplementary Table 1. Primers used to assess dinucleotides at the ligation junction**

The primers followed the format X_Y_FOR or X_Y_phos where X represents the nucleotide at the 3′-end and Y represents the nucleotide at the 5′-end of the ligation junction. 'phos' primers were already 5′ phosphorylated.

| Name | Sequence |
|---|---|
| C_C_FOR | CTGAAGCTCATCTGCACC |
| G_T_FOR | TCCAGGAGCGCACCATCTTC |
| T_C_FOR | CAAGGACGACGGCAACTAC |
| G_C_FOR | CATCGAGCTGAAGGGCATC |
| C_T_FOR | TTCAAGGAGGACGGCAACATCC |
| G_G_FOR | GGAATTCTCGAGTAAGGTTAACC |
| T_A_FOR | ATATCCGGAAGCTTGGCACTGG |
| G_A_FOR | AGCGGTATCAGCTCACTC |
| C_A_FOR | AAGTCAGAGGTGGCGAAACCC |
| A_A_FOR | AGCGTGGCGCTTTCTCATAGC |
| T_G_phos | GTAGGTCGTTCGCTCCAAGC |
| A_G_FOR | GTAAACTTGGTCTGACAGTTACC |
| A_T_FOR | TCTCAGCGATCTGTCTATTTCG |
| T_T_FOR | TCATCCATAGTTGCCTGAC |
| C_G_phos | GAGTTGCTCTTGCCCGGCGTC |
| A_C_phos | CACGGAAATGTTGAATACTC |

**Supplementary Table 2. mV_nicks and mNG_BbvCI plasmid sequences**

| Plasmid | Sequence |
|---|---|
| mV_nicks | GCTAGTGGTGCTAGCCCCGCGAAATTAATACGACTCACTATAGG<br>GTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGGTATACATA<br>TGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCAT<br>CCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGC<br>GTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGA<br>CCCTGAAGCTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGG<br>CCCACCCTCGTGACCACCCTCGGCTACGGCCTGCAGTGCTTCGC<br>CCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCG<br>CCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAG<br>GACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGG<br>GCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTT<br>CAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAAC<br>TACAACAGCCACAACGTCTATATCACCGCCGACAAGCAGAAGA<br>ACGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGA<br>CGGCGGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCC<br>ATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAG<br>CTACCAGTCCAAGCTGAGCAAAGACCCCAACGAGAAGCGCGAT<br>CACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCT<br>CGGCATGGACGAGCTGTACAAGTAATGACCTCAGCGGATCCGC<br>TCTTCCGGGAATTCTCGAGTAAGGTTAACCTGCAGGAGGCCTTT<br>AATTAAGGTGGTGCGGCCGCGCTAGCGGTCCCGGGGGATCGAT<br>CCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTG<br>CCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAA |

| | |
|---|---|
| | CGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGG AAGCTTGGCACTGGCCGACCGGGGTCGAGCACTGACTCGCTGC GCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAG GCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAA AGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTA AAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCT GACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAA ACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAG CTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATA CCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAG CTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCA AGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGC GCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGCTAAGACA CGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCA GAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTG GCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCG CTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCT TGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGT TTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAA GATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGA AAACTCACAGATCCGGGATTTTGGTCATGAGATTATCAAAAAGG ATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATC AATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAAT GCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTT CATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATA CGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCG AGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGC CAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATC CGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAA GTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCT ACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATT CAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCA TGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTT GTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGC AGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTT TTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGT GTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGAT AATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGG AAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGT TGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCT TCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAAC AGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACG GAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAG CATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAAT GTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCC CGAAAAGT |
| mNG_BbvCI | TCGACCGCCAATTCAATATGGCGTATATGGACTCATGCCAATTC AATATGGTGGATCTGGACCTGTGCCAATTCAATATGGCGTATAT GGACTCGTGCCAATTCAATATGGTGGATCTGGACCCCAGCCAAT TCAATATGGCGGACTTGGCACCATGCCAATTCAATATGGCGGAC |

| | |
|---|---|
| | CTGGCACTGTGCCAACTGGGGAGGGGTCTACTTGGCACGGTGCC<br>AAGTTTGAGGAGGGGTCTTGGCCCTGTGCCAAGTCCGCCATATT<br>GAATTGGCATGGTGCCAATAATGGCGGCCATATTGGCTATATGC<br>CAGGATCAATATATAGGCAATATCCAATATGGCCCTATGCCAAT<br>ATGGCTATTGGCCAGGTTCAATACTATGTATTGGCCCTATGCCA<br>TATAGTATTCCATATATGGGTTTTCCTATTGACGTAGATAGCCCC<br>TCCCAATGGGCGGTCCCATATACCATATATGGGGCTTCCTAATA<br>CCGCCCATAGCCACTCCCCCATTGACGTCAATGGTCTCTATATA<br>TGGTCTTTCCTATTGACGTCATATGGGCGGTCCTATTGACGTATA<br>TGGCGCCTCCCCCATTGACGTCAATTACGGTAAATGGCCCGCCT<br>GGCTCAATGCCCATTGACGTCAATAGGACCACCCACCATTGACG<br>TCAATGGGATGGCTCATTGCCCATTCATATCCGTTCTCACGCCCC<br>CTATTGACGTCAATGACGGTAAATGGCCCACTTGGCAGTACATC<br>AATATCTATTAATAGTAACTTGGCAAGTACATTACTATTGGAAG<br>TACGCCAGGGTACATTGGCAGTACTCCCATTGACGTCAATGGCG<br>GTAAATGGCCCGCGATGGCTGCCAAGTACATCCCCATTGACGTC<br>AATGGGGAGGGGCAATGACGCAAATGGGCGTTCCATTGACGTA<br>AATGGGCGGTAGGCGTGCCTAATGGGAGGTCTATATAAGCAAT<br>GCTCGTTTAGGGAACCGCCATTCTGCCTGGGGACGTCGGAGCAA<br>GCTTGATTTAGGTGACACTATAGACTTGTTCTTTTTGCAGGATCT<br>ACCATGGTGAGCAAGGGCGAGGAGGATAACATGGCCTCTCTCC<br>CAGCGACACATGAGTTACACATCTTTGGCTCCATCAACGGTGTG<br>GACTTTGACATGGTGGGTCAGGGCACCGGCAATCCAAATGATG<br>GTTATGAGGAGTTAAACCTGAAGTCCACCAAGGGTGACCTCCA<br>GTTCTCCCCCTGGATTCTGGTCCCTCATATCGGGTATGGCTTCCA<br>TCAGTACCTGCCCTACCCTGACGGGATGTCGCCTTTCCAGGCCG<br>CCATGGTAGATGGCTCCGGATACCAAGTCCATCGCACAATGCAG<br>TTTGAAGATGGTGCCTCCCTTACTGTTAACTACCGCTACACCTAC<br>GAGGGAAGCCACATCAAAGGAGAGGCCCAGGTGAAGGGGACT<br>GGTTTCCCTGCTGACGGTCCTGTGATGACCAACTCGCTGACCGC<br>TGCGGACTGGTGCAGGTCGAAGAAGACTTACCCCAACGACAAA<br>ACCATCATCAGTACCTTTAAGTGGAGTTACACCACTGGAAATGG<br>CAAGCGCTACCGGAGCACTGCGCGGACCACCTACACCTTTGCCA<br>AGCCAATGGCGGCTAACTATCTGAAGAACCAGCCGATGTACGT<br>GTTCCGTAAGACGGAGCTCAAGCACTCCAAGACCGAGCTCAAC<br>TTCAAGGAGTGGCAAAAGGCCTTTACCGATGTGATGGGCATGG<br>ACGAGCTGTACAAGGGATCTGGATCCCATCGATTCGAATTCAAG<br>GCCTCTCGAGCCTCTAGAACTATAGTGAGTCGTATTACGTAGAT<br>CCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAA<br>CTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGAT<br>GCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGT<br>TAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGG<br>AGGTGTGGGAGGTTTTTTAATTCGCGGCCGCGGCGCCAATGCAT<br>TGGGCCCGGTACCCAGCTTTTGTTCCCTTTAGTGAGGGTTAATTG<br>CGCGCTTGGCGTAATCCCTCAGCATGGTCATAGCTGTTTCCTGT<br>GTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCG<br>GGAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTA<br>ACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGG<br>GAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCG<br>GGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCT |

| | |
|---|---|
| | CACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTAT<br>CAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGG<br>GGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAA<br>GGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATA<br>GGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGT<br>CAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGT<br>TTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGC<br>CGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTG<br>GCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTA<br>GGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTC<br>AGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCC<br>AACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTG<br>GTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGA<br>GTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAG<br>TATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAA<br>AGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAG<br>CGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAA<br>AAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGAC<br>GCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAG<br>ATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAAT<br>GAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCT<br>GACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGAT<br>CTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTA<br>GATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTG<br>CAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCA<br>GCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTC<br>CTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGG<br>AAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTT<br>GTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGG<br>TATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTA<br>CATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGT<br>CCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACT<br>CATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATC<br>CGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCAT<br>TCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCG<br>TCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAG<br>TGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGG<br>ATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGC<br>ACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGG<br>GTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAAT<br>AAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTC<br>AATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGA<br>TACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCC<br>GCGCACATTTCCCCGAAAAGTGCCACCTAAATTGTAAGCGTTAA<br>TATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATT<br>TTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAA<br>AAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAAC<br>AAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGC<br>GAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATC<br>ACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAA |

| | |
|---|---|
| | ATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGG<br>AAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAA<br>AGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTG<br>CGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGG<br>CGCGTCCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGC<br>GATCGGTGCGGGCCTCTTCGCTATTACGCCAG |

**Supplementary Table 3. Primers used for PCR, RT-qPCR and NEEL**
Underlined sequences are the T7 promoter and TATA box. Bolded sequences are the upstream and downstream BRE and Inr elements. X represents the amino-C6-dT modifications.

| Name | Sequence |
|---|---|
| mV nick T7T FRW | TACAAGTAATGACCTCAGCGGATCCGCTCTTCCGGGAATTCTCGAGTAAGG |
| DHFR REV T7S GFP | ACAGCTCCTCGCCCTTGCTCACCATATGTATACCTCCTTCTTAAAGTTAAAC |
| GFP FRW T7S | CGGCATGGACGAGCTGTACAAGTAATGAGGATCCCGGGAATTCTCGAGTA |
| mV nick T7T REV | AATTCCCGGAAGAGCGGATCCGCTGAGGTCATTACTTGTACAGCTCG |
| mNG_BbvCI_FOR1 | GCTTGGCGTAATCCCTCAGCATGGTCATAGCTG |
| mNG_BbvCI_REV2 | CAGCTATGACCATGCTGAGGGATTACGCCAAGC |
| mNG_nick_mutagenesis_REV1 | CGAGAGGCCTTGAATTCGAATCGATG |
| mNG_nick_mutagenesis_FOR2 | CATCGATTCGAATTCAAGGCCTCTCG |
| mNG_qPCR_FOR | CGTGTTCCGTAAGACGGAGC |
| mNG_RT-qPCR_REV | CCTTGTACAGCTCGTCCATGC |
| GAPDH_FOR_Origene | GTCTCCTCTGACTTCAACAGCG |
| GAPDH_REV_IDT | TCCACCACCCTGTTGCTGTA |
| T7_eLong | GAAATTAATACGACTCACTATAGGGTCTAG |
| T7_7amines | GAAATXAAXACGACXCACXAXAGGGXCXAG |
| mNG_9amines_46b | **GGXC**XAXAXAAG**CAAXGCX**CGTTTAGGGAACCG**CCATXCX**GCCXGG |

**Supplementary Table 4. Primers used to synthesise the backbones and inserts for homologous recombinations**

| PCR | DNA template | For primer | Rev Primer |
|---|---|---|---|
| mV_nicks_BB | mVenus_CT | mV nick T7T FRW | DHFR REV T7S GFP |
| mV_nicks_insert | mVenus_CT | GFP FRW T7S | mV nick T7T REV |
| mNG_BbvCI_BB | pCS2-mNG-C plasmid | mNG_BbvCI_FOR1 | mNG_nick_mutagenesis_REV1 |
| mNG_BbvCI_insert | pCS2-mNG-C plasmid | mNG_BbvCI_REV2 | mNG_nick_mutagenesis_FOR2 |

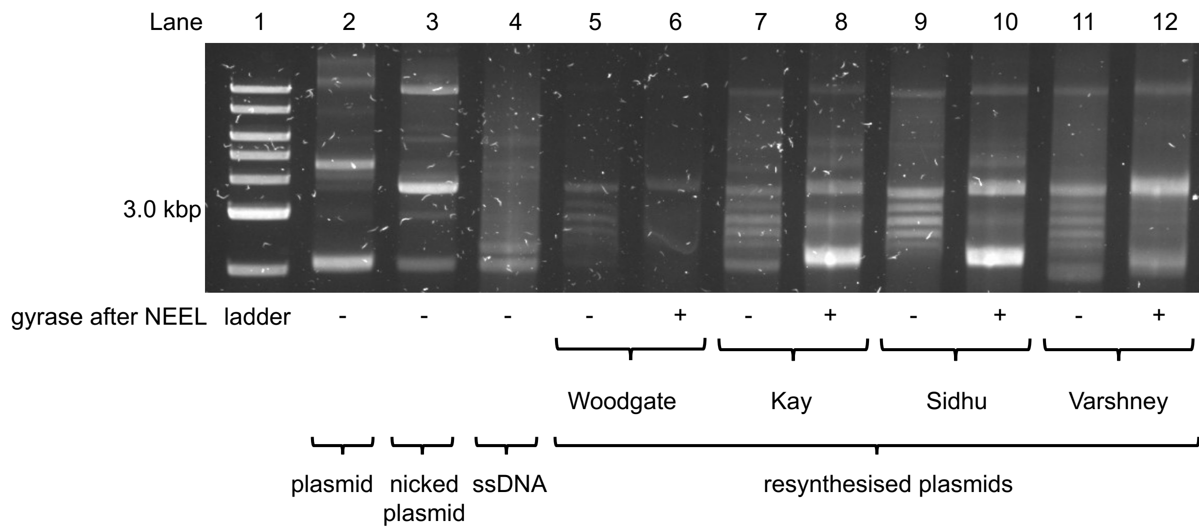
**Supplementary Table 5. Backbone and insert combinations for homologous recombinations**

| Plasmid | Backbone | Insert |
|---|---|---|
| mV_nicks | mV_nicks_BB | mV_nicks_insert |
| mNG_BbvCI | mNG_BbvCI_BB | mNG_BbvCI_insert |

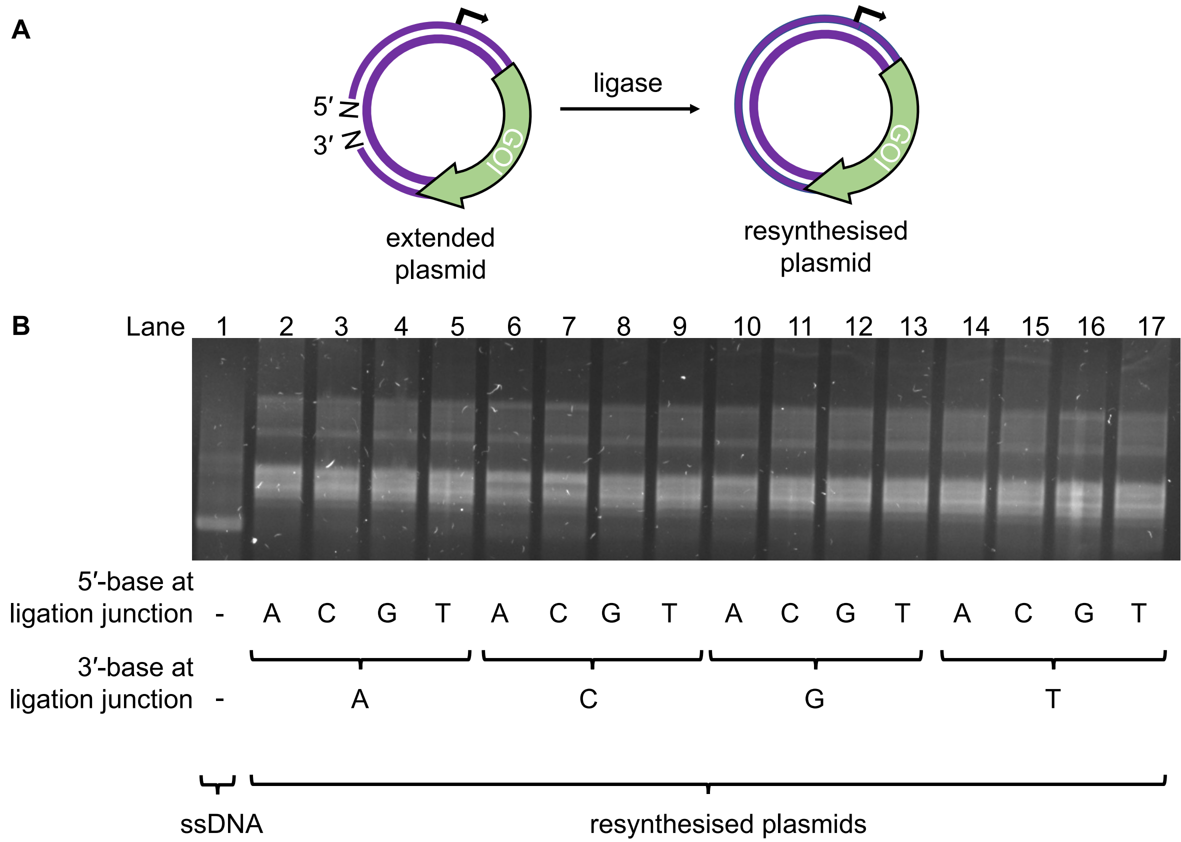
**Supplementary Table 6. HPLC Purification of the PCB oligos**

| Promoter | Number of modifications | Collection period/ mins | Increments/ mins | Fractions pooled |
|---|---|---|---|---|
| T7 | 7 | 24.0-25.5 | 0.15 | 5-7 |
| CMV | 9 | 24.0-25.5 | 0.15 | 3-5 |

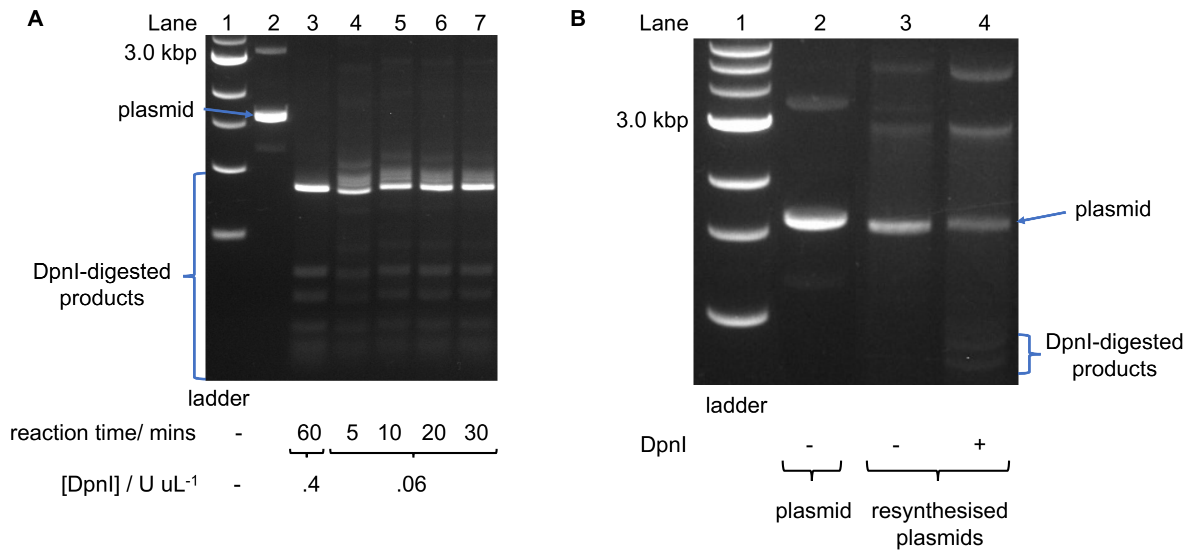
**Supplementary Data**



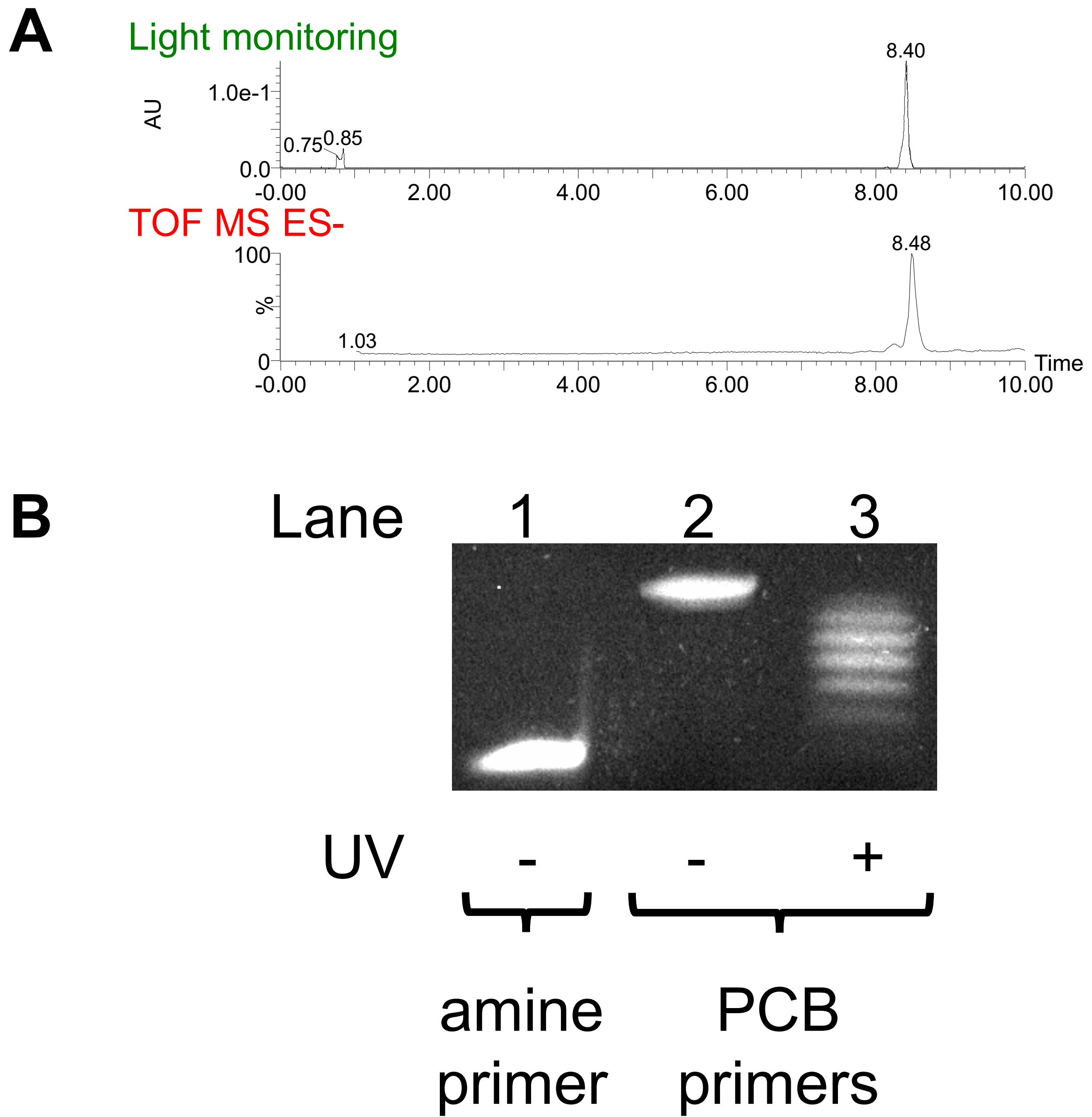

**SI Figure 1. Different Kunkel methods were employed to resynthesise the plasmid.** Kunkel mutagenesis protocols from the Woodgate,[1] Kay,[2] Sidhu,[3] and Varshney[4] laboratories were used to regenerate the plasmid. After ligation, the different plasmids were reacted with gyrase to test for ligation.



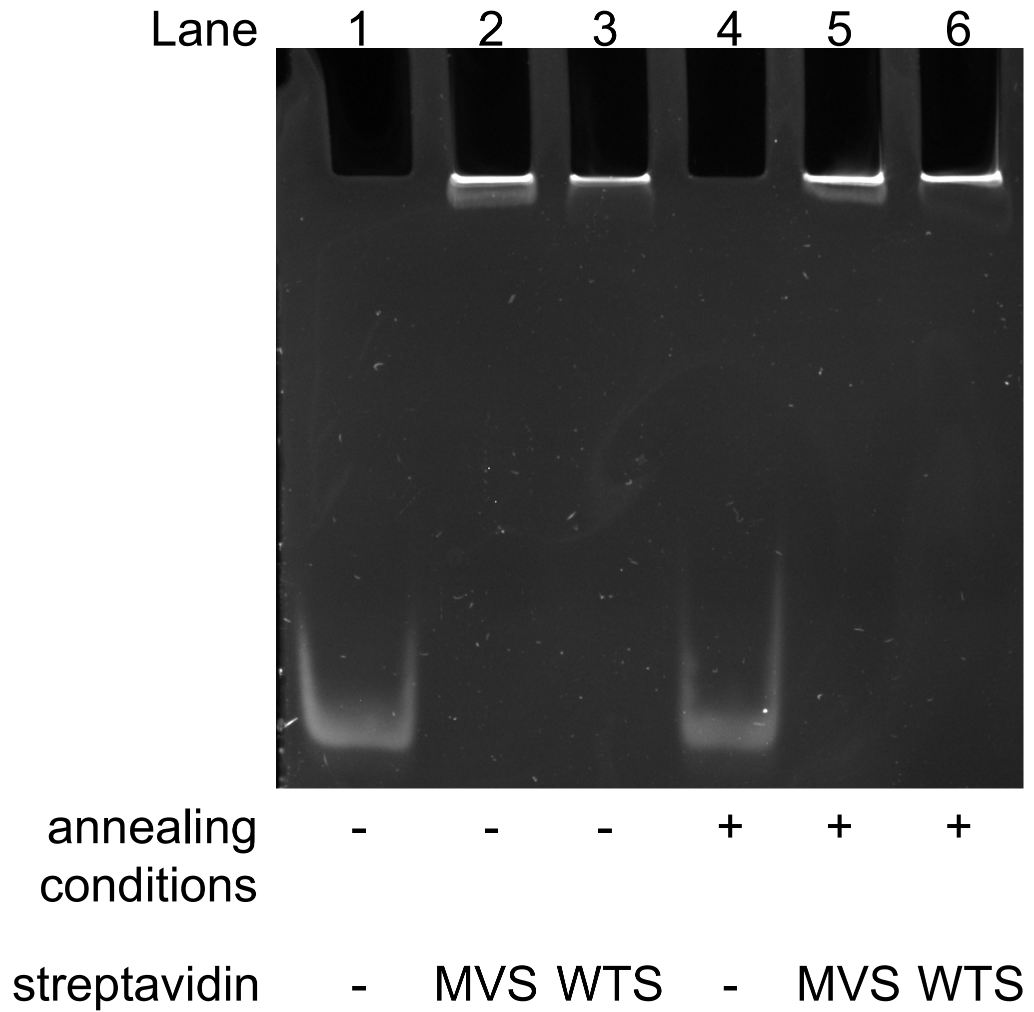

**SI Figure 2. NEEL with all possible dinucleotide combinations at the ligation junction. A.** Depiction of the reaction in (**B**). **B.** Different primers with varying $T_m$, GC content, and lengths were used for NEEL. Ligation at the different ligation junctions was equally effective.



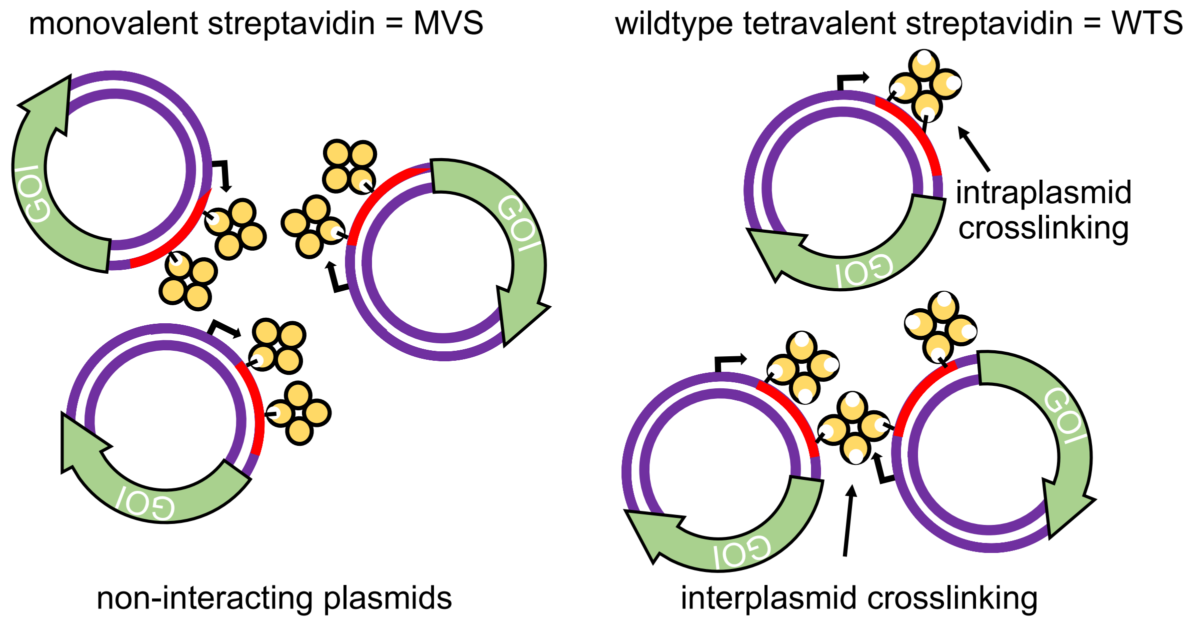

**SI Figure 3. DpnI removal of unreacted plasmids. A.** Mild DpnI conditions were optimised with native mVenus plasmid. **B.** When resynthesised plasmids were reacted with DpnI faint degraded plasmid bands appeared whilst the majority of the resynthesised plasmids were intact.



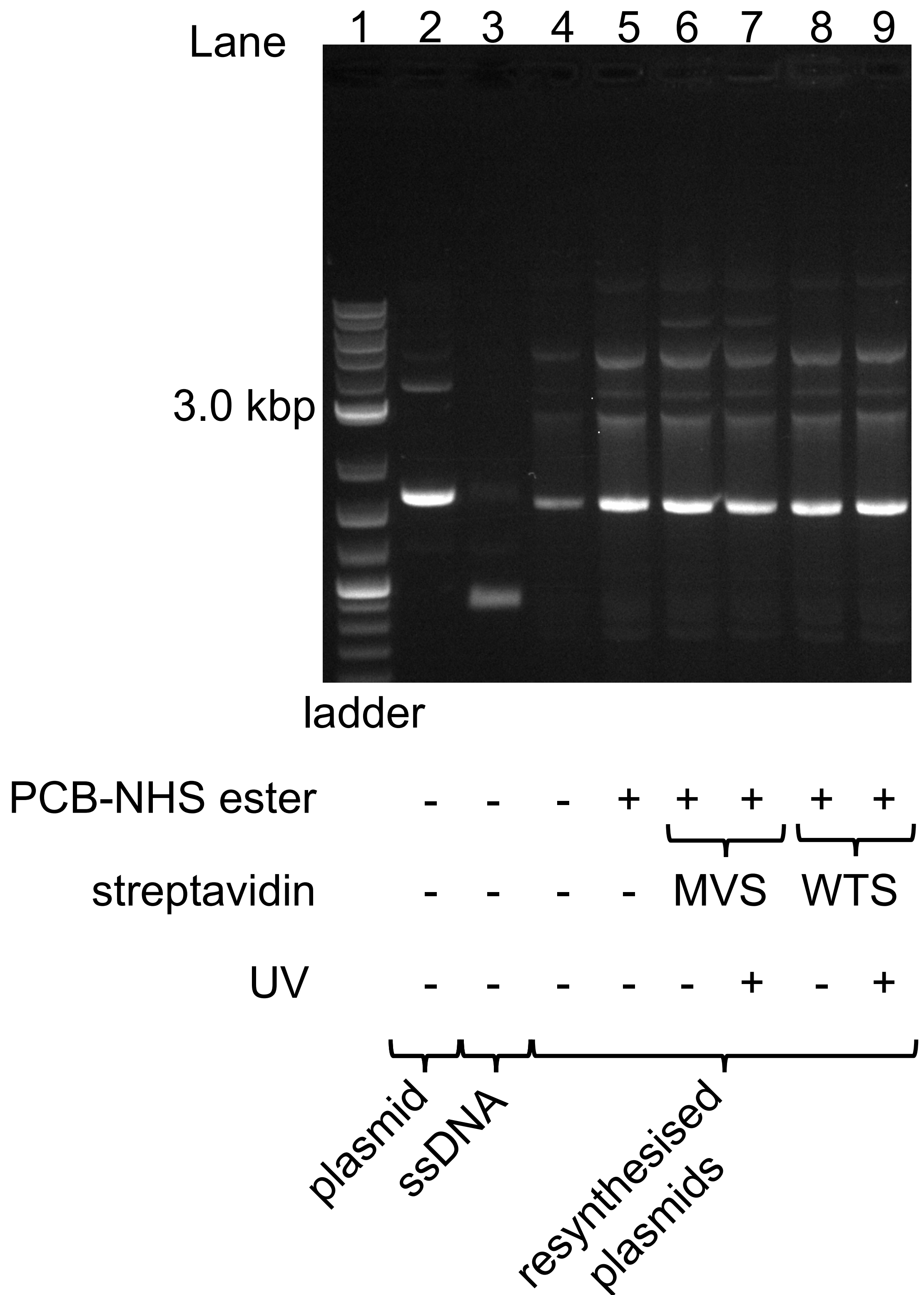

**SI Figure 4. Confirming synthesis of the T7 PCB oligo. A.** LC-MS and **B.** Denaturing PAGE were used to confirm PCB attachment. MS gave a mass of 15398, which corresponded to the 7-PCBs oligo.



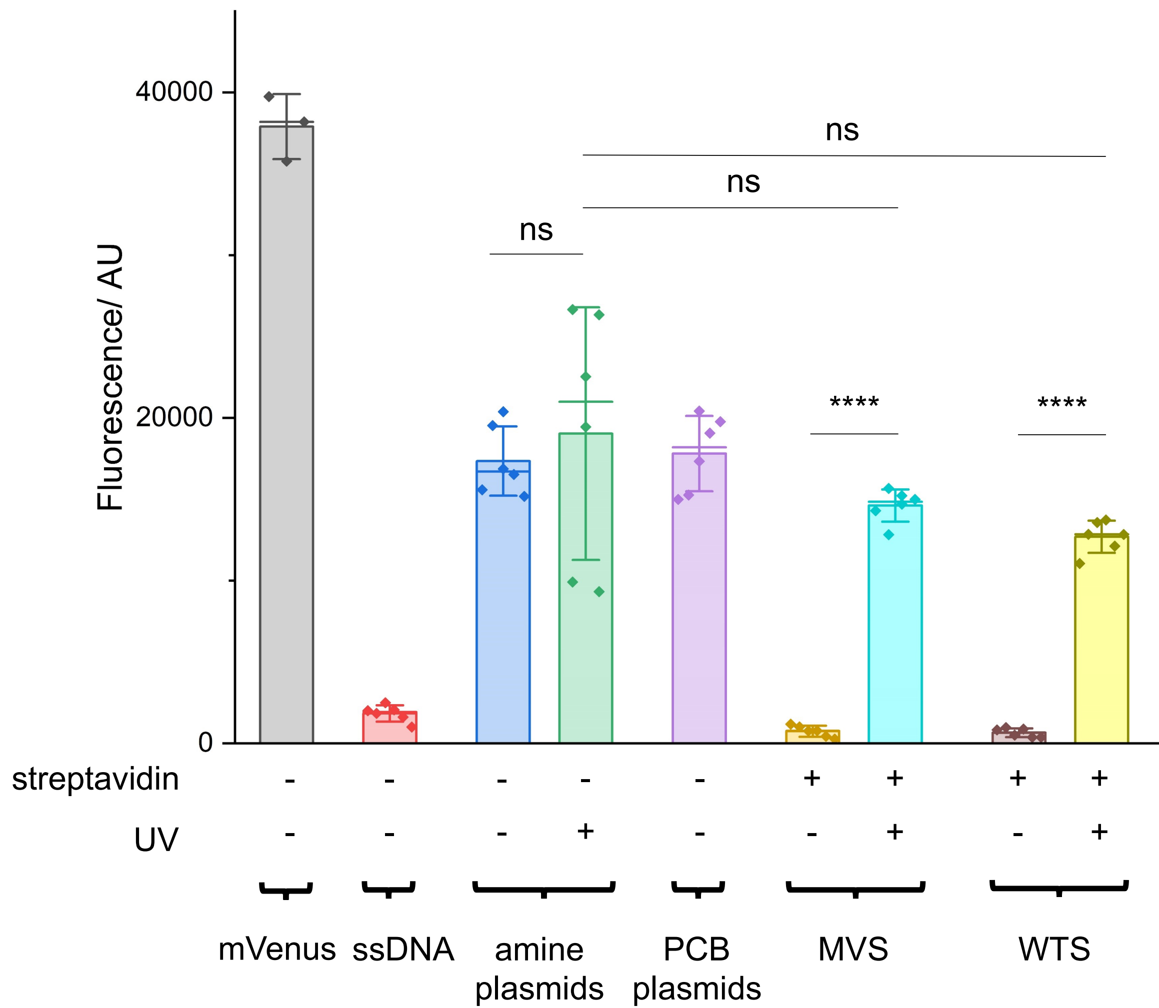

**SI Figure 5. PCB oligo was unaffected by annealing conditions.** PCB oligo was bound to either monovalent or wildtype tetravalent streptavidin (MVS and WTS, respectively) and incubated in annealing conditions.



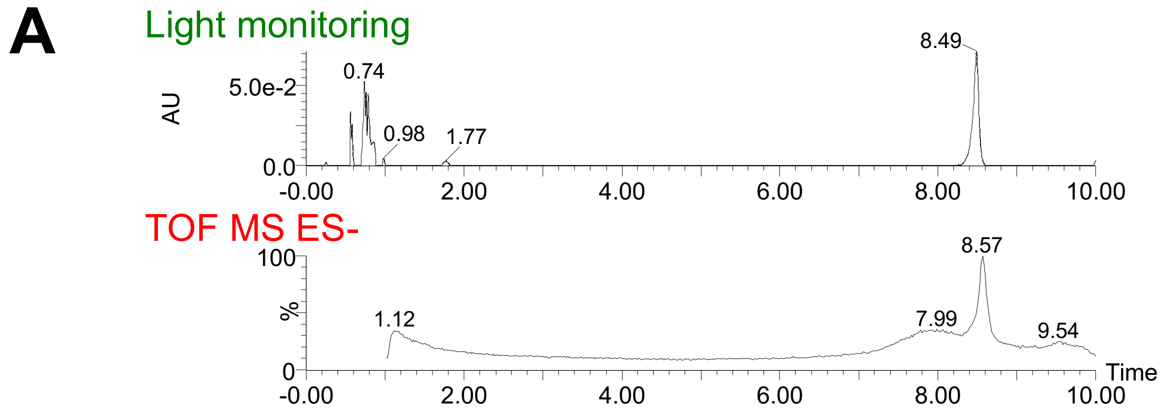

**SI Figure 6. Comparison between MVS and WTS LA-plasmids.** With only a single active subunit, MVS LA-plasmids do not interact. WTS has four active subunits, which may give rise to intra- and interplasmid crosslinking.

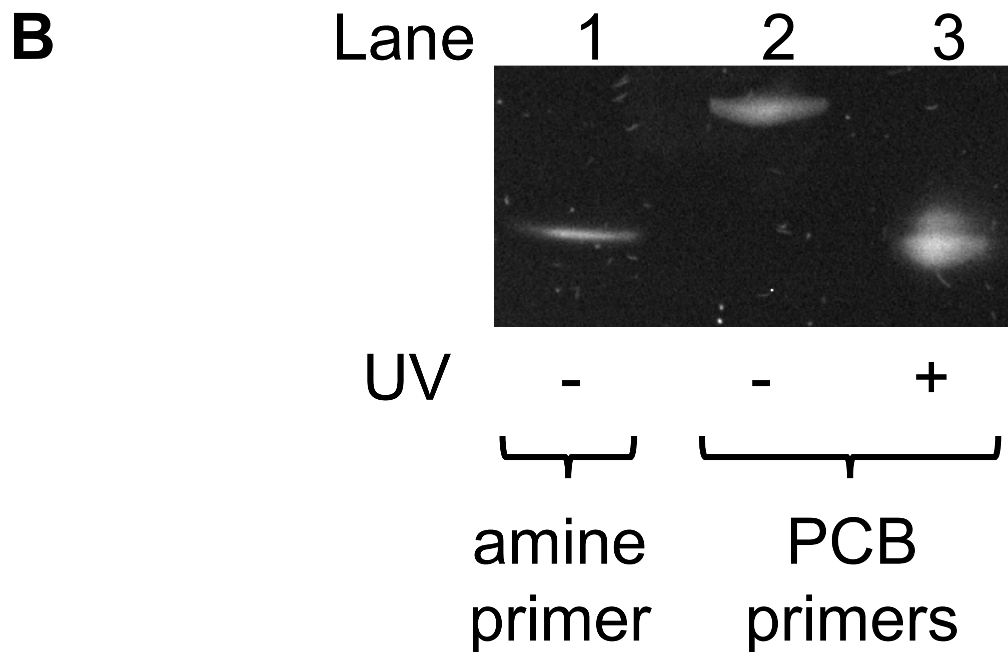



**SI Figure 7. Negative controls without amino modifications.** Plasmids were resynthesised using a regular primer without amino modifications. The absence of primary amine modifications prevented PCB conjugation and thereby streptavidin binding.

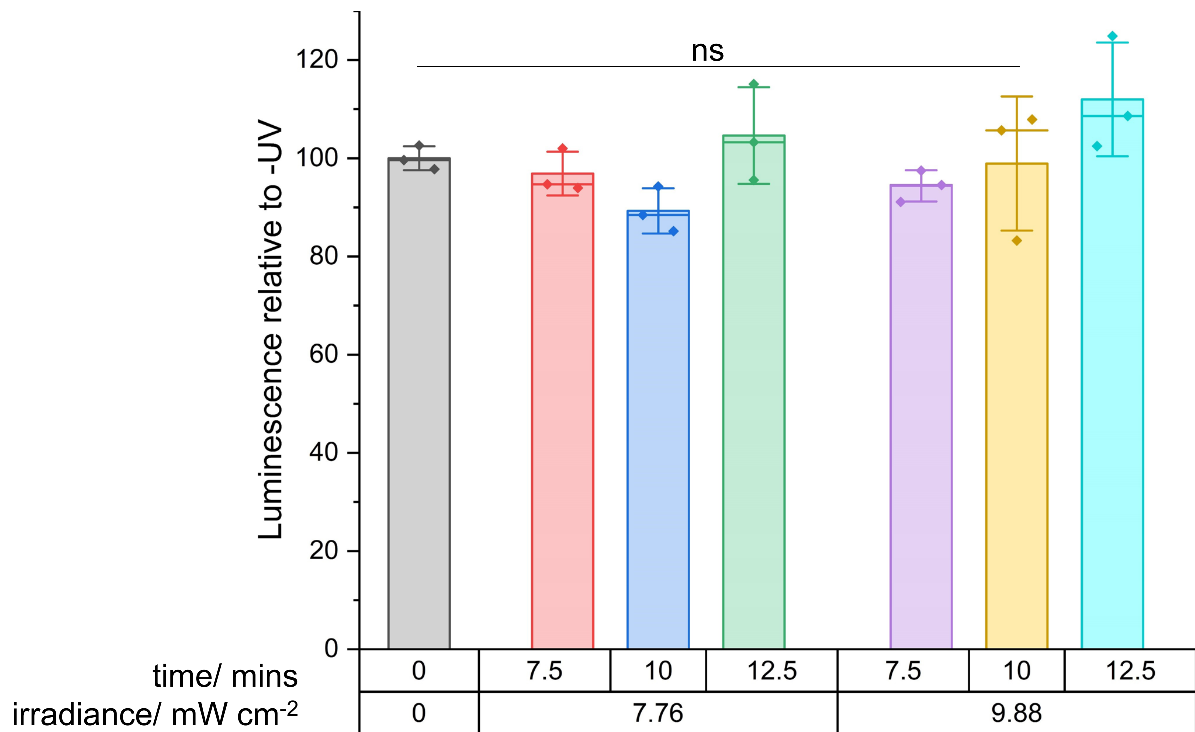



**SI Figure 8. CFPS of T7 NEEL plasmids including the native plasmid.** Plasmids containing the T7 promoter were prepared with NEEL and assessed using CFPS. Native plasmid and NTC were technical triplicates ($n$ = 3). Replicates, measurements, and statistical analysis were similar to those in **Figure 4B**.

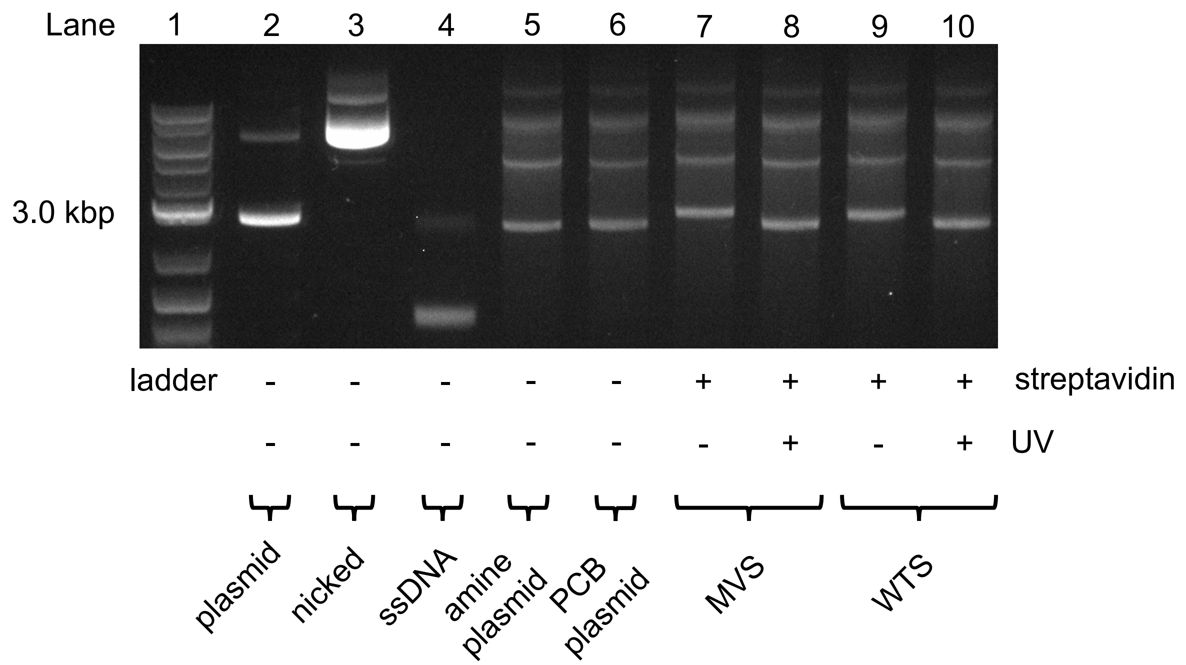


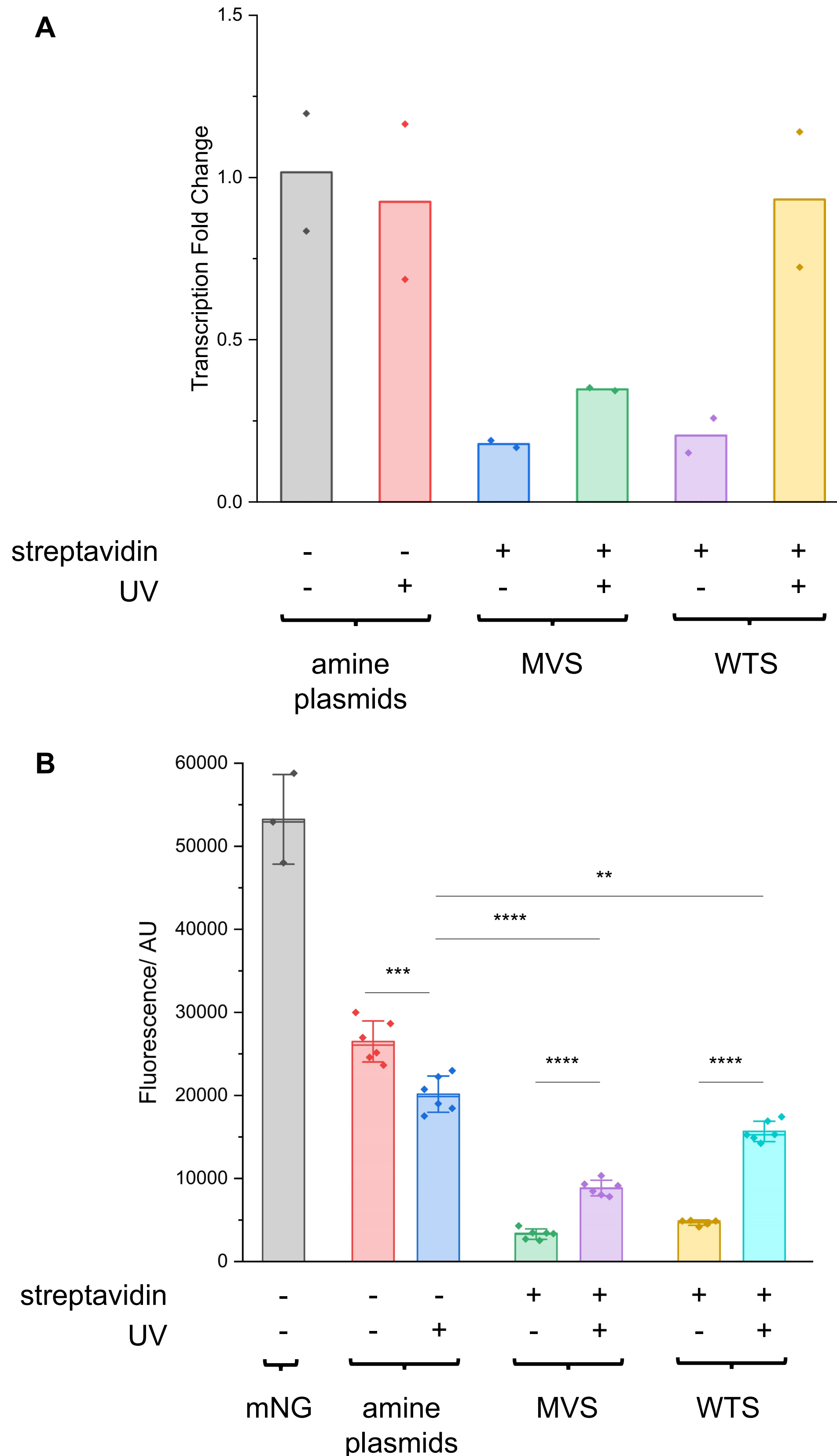

**SI Figure 9. Confirming synthesis of the CMV PCB oligo A.** LC-MS and **B.** Denaturing PAGE were used to confirm PCB attachment. MS gave a mass of 22076, which corresponded to the 9-PCBs oligo.



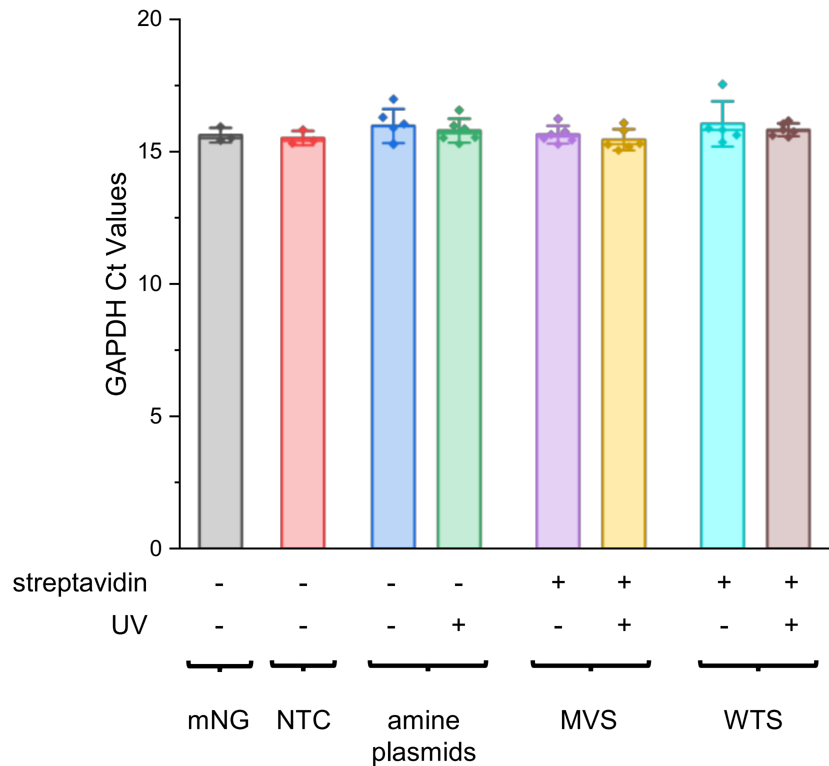

**SI Figure 10. Cell viability assay with optimised UV settings for transfection.** Cell viability assay with various UV settings. The conditions employed, 9.88 mW cm$^{-2}$ for 10 mins, had minimal damage to cells. Error bars showed standard deviations of technical triplicates ($n = 3$). Two-tailed, unpaired Student's t-test was performed. ns = non-significant ($P > .05$).




**SI Figure 11. CMV LA-plasmids and their uncaging.** The PCB plasmids were bound to streptavidins and uncaged using the optimised UV settings in **SI Figure 10**.




**SI Figure 12. RT-qPCR and fluorescence measurements of CMV NEEL plasmids, including the native plasmid.** Plasmids containing the CMV promoter were prepared with NEEL and assessed using **A.** RT-qPCR and **B.** Fluorescent plate reader. Replicates, measurements, and statistical analysis were similar to those in **Figure 5**.




**SI Figure 13. GAPDH transcription levels after transfection with modified plasmids.** Plasmids containing the photocaged CMV promoter were transfected into HEK293T cells and irradiated with UV. RT-qPCR was performed and the Ct values of the GAPDH gene were compared. Error bars show standard deviations. Two-tailed, unpaired Student's t-test was performed. ns = non-significant ($P > .05$); **$.001 \leq P \leq .01$; ***$.0001 \leq P \leq .001$ and ****$P \leq .0001$. 'mNG' and 'NTC' conditions were technical triplicates ($n$ = 3), and all other conditions were biological duplicates of technical triplicates ($n$ = 6) except for the 'WTS-UV' condition ($n$ = 5 due to sample loss).

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